Supporting Information

Supporting Text. Definitions of terms used for dental development assessment.

accentuated line: A pronounced internal line corresponding to the position of the developing enamel or dentine front that relates to a stressor experienced during tooth development (as opposed to an intrinsic rhythm). The **neonatal line** found in teeth forming during birth is the most common example. Because accentuated lines form in teeth developing at the same time, they may be used to register, or cross-match teeth. Thus counts of successive developmental time can be continued from one tooth to another when equivalent accentuated lines (or **hypoplasias**) can be identified and registered.

Andresen lines: Long-period lines (> daily) in dentine representing the successive positions of the dentine-forming front, which may be used to assess **root formation time** as they correspond to **periradicular bands** on the root surface, and are temporally equivalent to **Retzius lines** in enamel and **perikymata** on the crown surface. Illustrated in SI Fig 14.

coronal extension rate: See **extension rate**.

cross-striations: Daily **short-period lines** along enamel prisms running at approximate right angles to prism axes. Cross-striations represent a 24-hour cycle of ameloblast activity. The distance between adjacent cross-striations yields the enamel **daily secretion rate**, and counts of these lines between successive **long-period lines** yield the **long-period line periodicity**. Illustrated in SI Fig. 13.

crown formation time: The time it takes to develop the tooth crown, which is typically assessed through counts and measurements of **incremental features**. The most common method involves summing cuspal and lateral enamel formation times. Cuspal enamel formation time is determined by division of the **cuspal enamel thickness** by the **daily secretion rate**. A correction factor is sometimes employed to increase the linear thickness to compensate for the three-dimensional curved path of enamel-forming cells. Lateral enamel formation time is determined by multiplication of the total number of **long-period lines** by the **long-period line periodicity**. For multi-cusped teeth, the total crown formation time is typically longer than the cusp-specific crown formation time (employed in the current study), as each cusp may initiate and complete formation at different ages. Also see **enamel development**.

cuspal enamel thickness: Linear measurement of enamel thickness from the dentine horn tip to the position of the first **long-period line** at the tooth surface, which is a two-dimensional proxy for the path of cuspal enamel-forming cells. The position of the first-formed long-period line is fairly invariant, thus this value can be determined from micro-CT scans to assess the cuspal enamel formation time with reasonable accuracy.

daily secretion rate: The rate of enamel or dentine secretion, which may be measured from the spacing between successive **short-period lines** (or **long-period lines** of known periodicity) along enamel prisms or dentine tubules. Since the average cuspal enamel daily secretion rate is fairly invariant within a taxon, a mean value can be used to approximate cuspal formation time in teeth for which the daily secretion rate is unknown.

dentine development: Dentine is formed when dentine-forming cells (odontoblasts) secrete a collagenous matrix (predentine) which undergoes mineralization to form primary dentine. The path of these cells is recorded by fine processes that remain behind in dentine tubules. Dentine formation begins at the future dentine horn, underlying the future cusp tip, and progresses inward through secretion and downward through extension until it reaches the apex of the root.

enamel development: Enamel is formed when enamel-forming cells (ameloblasts) secrete enamel matrix proteins that mineralize into long thin bundles of crystallites known as enamel prisms. As the secretory cells progress outward towards the future tooth surface, additional cells are activated through extension (in a cervical direction) until the forming-front reaches the cervix of the crown, where it ceases secretion.

eruption: The process of tooth crown emergence; a tooth must past the bone margin (alveolar eruption) and the gum (gingival eruption) in order to emerge into the oral cavity, and eventually into functional occlusion. Most studies of recent humans report gingival eruption ages from living individuals, while alveolar eruption is easier to assess from skeletonized individuals.

extension rate: The rate at which secretory cells are activated to begin hard tissue secretion. Extension in the tooth crown proceeds from the future dentine horn tip to the future cervix, and both enamel- and dentine-forming cells extend at an equivalent rate. Calculation of the coronal extension rate, representing an average rate over the whole formation period, is determined from division of the enamel-dentine junction length by the crown formation time specific to that cusp. Upon completion of crown formation, dentine-forming cells continue to extend apically, beginning root formation. Root extension rates may be determined from counts or measurements of **long-period lines** (of known periodicity) along the root surface.

hypoplasia: Structural feature formed by a stressor experienced during development, which results in an anomalous depression (furrow or pit) on the surface of a tooth. These external features are often associated with **accentuated lines** internally, and can be used to cross-register teeth forming at the same time.

incremental features: Microscopic markings that represent intrinsic temporal rhythms in hard tissue secretion, which include **long-period lines** (e.g., **Retzius lines** in enamel) and **short-period lines** (e.g., **cross-striations** or **laminations** in enamel).

initiation age: The age at which a tooth begins hard tissue formation (crown calcification). This can be determined for first molars (M1s) by identification of the neonatal line and calculation of the prenatal formation from **short-period lines** in enamel or dentine, which yield the number of days before birth that a tooth began forming. For most permanent teeth, which initiate formation after birth, initiation age must be determined by cross-matching (or registering) teeth with one another via **accentuated lines** or **hypoplasias** and an event of known age (e.g., birth or death).

laminations: Daily **short-period lines** that run parallel to the developing enamel front (and **Retzius lines**), which are temporally and structurally equivalent to **cross-striations** but exhibit an oblique orientation relative to enamel prism long axes. By varying slice thickness with virtual histology, laminations appear as three-dimensional alignments of isochronous cross-striations across enamel prisms, which may be used for assessment of the **long-period line periodicity**. Illustrated in SI Figs. 12 & 13.

long-period lines: Incremental lines in enamel or dentine representing the successive positions of the developing cellular front. The term long-period refers to the intrinsic temporal repeat interval that is greater than one day (in contrast to daily **short-period lines**). Long-period lines in enamel are known as **Retzius lines** (SI Fig. 12), which terminate on the tooth surface as **perikymata** (SI Movie 1). Long-period lines in dentine are known as **Andresen lines** (SI Fig. 14), which terminate on the root surface as **periradicular bands**. The temporal repeat interval is referred to as the **long-period line periodicity.**

long-period line periodicity: Number of days between successive **long-period lines**, determined by counting daily **short-period lines** between intervals. This integer is assessed by counting daily **cross-striations** or **laminations** between pairs of **Retzius lines**, which may only be determined from the inside of a tooth, necessitating physical sectioning or virtual histology. The value for long-period line periodicity is consistent within all teeth belonging to an individual's dentition, but may vary among individuals within a species. Illustrated in SI Figs. 12 & 13.

neonatal line: Accentuated line found in enamel and dentine of teeth developing just prior to, during, and after birth, which allows developmental time to be registered to chronological age. Illustrated in Fig. 1 & SI Fig 1.

perikymata: **Long-period lines** (> daily) that manifest as external ridges and troughs encircling the tooth crown, which are formed by **Retzius lines** as they reach the enamel surface. Perikymata are analogous to **periradicular bands** in dentine. The region of the crown that exhibits perikymata is called the lateral enamel.

periodicity: See **long-period line periodicity**.

periradicular bands: Long-period lines (> daily) that manifest as external ridges and troughs encircling the tooth root, and are formed by **Andresen lines** as they reach the root surface.

Retzius lines: **Long-period lines** (> daily) in enamel that represent the position of the developing cellular front at successive points in time. They manifest on the tooth surface as **perikymata**, and are analogous to the **Andresen lines** in dentine. Illustrated in SI Fig. 12.

root extension rate: See **extension rate.**

root formation time: The time it takes to develop the tooth root, which is typically assessed through counts and measurements of **incremental features**. In practice it is difficult to image a complete series of incremental features in a fully-formed root, so this is typically undertaken in teeth that are still forming their roots at the time of death. For incomplete teeth, the root formation time may be determined by division of the dentine tubule length by the **daily secretion rate**, or by multiplication of the number of **long-period lines** by the **long-period line periodicity** specific to that individual. Also see **dentine development**.

short-period lines: Incremental lines in enamel or dentine representing the daily secretion of the developing cellular front. The term short-period refers to the circadian or daily temporal repeat interval (in contrast to **long-period lines** that repeat over multiple days). Short-period lines in enamel are known as **cross-striations** and **laminations** (SI Fig. 12 & 13), which may be used to determine the **daily secretion rate**. Short-period lines in dentine are known as **von Ebner's lines.**

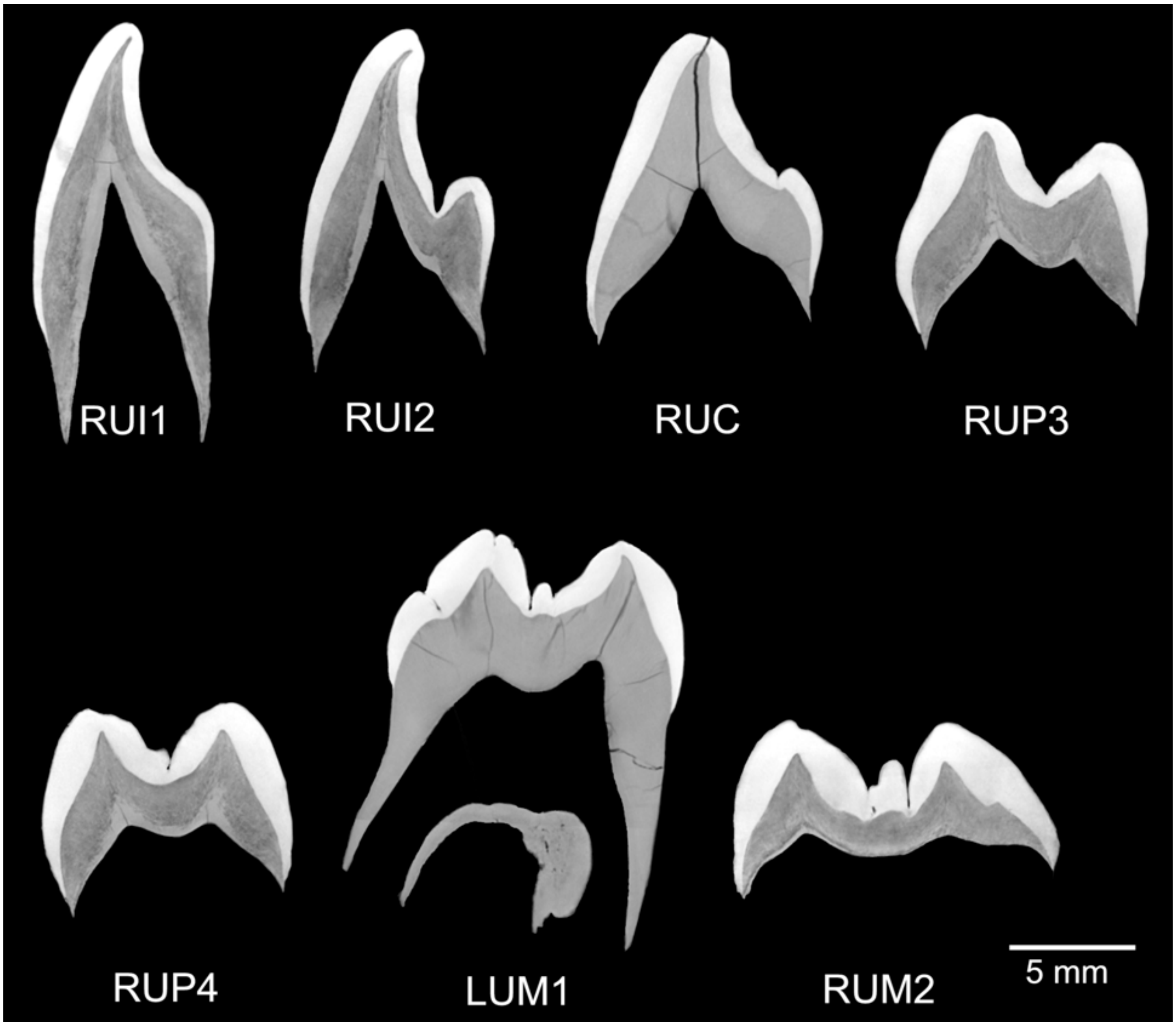
von Ebner's lines: Daily **short-period lines** in dentine that reflect a 24-hour cycle of dentine secretion, and are temporally equivalent to **cross-striations** and **laminations** in enamel. As it is difficult in practice to image these lines to determine the **daily secretion rate**, measurements of **Andresen lines** are generally preferred when the **long-period line periodicity** is known.

Supporting Figures

SI Figure 1. Neonatal line (dotted line) in the Krapina Maxilla C Neanderthal's maxillary first molar (M1) enamel and dentine.

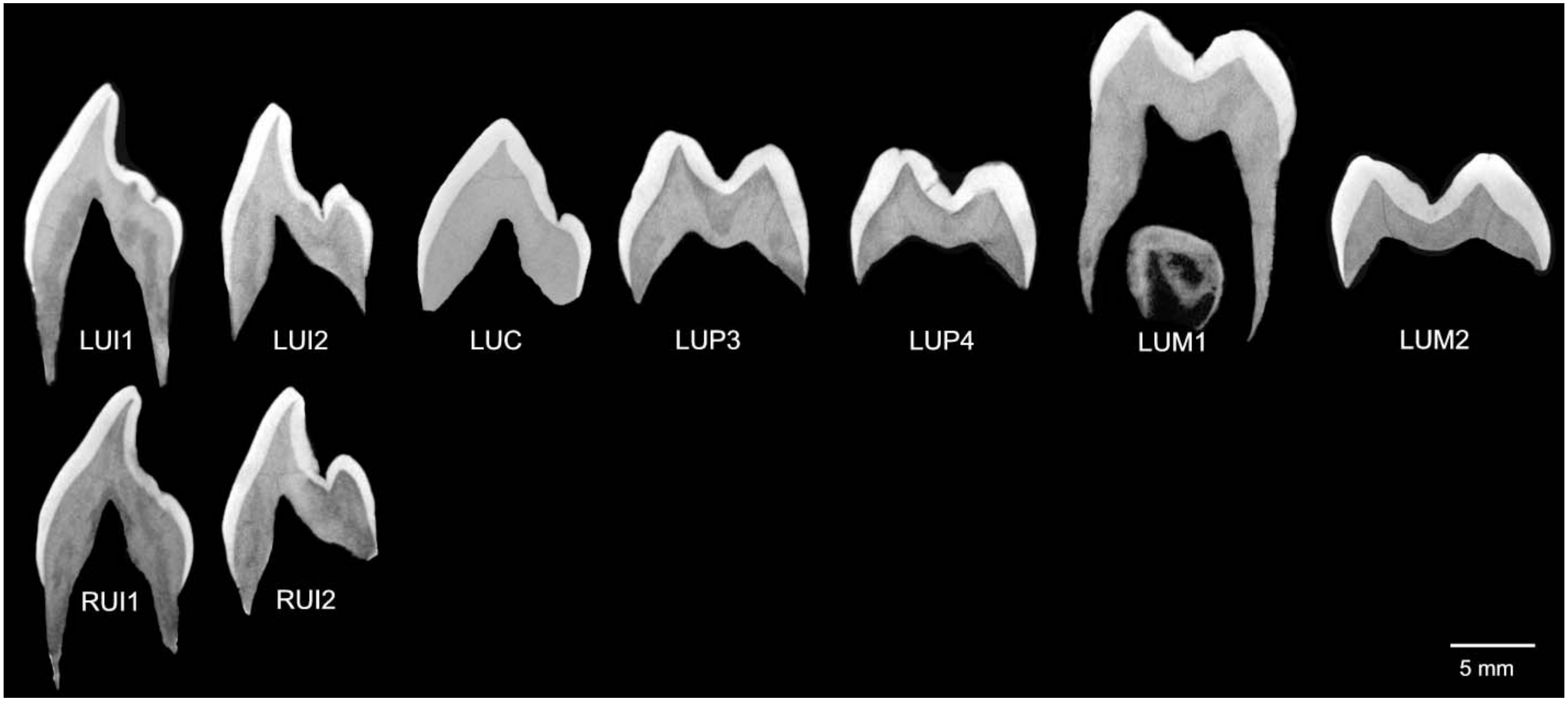
Enamel and dentine are separated by the enamel-dentine junction (dark boundary that arcs upward toward the center of the enamel); enamel is above, and dentine is below.
Neonatal lines are consistently found in Neanderthal molars within approximately 60 micrometers of the dentine horn tip, including maxillary M1s from Scladina (1), Engis 2, Dederiyeh 1 (2), Krapina Maxilla B, and Krapina Max C (above), and mandibular M1s from Gibraltar 2 and La Chaise (3).

SI Figure 2. Virtual overview of the La Quina H18 Neanderthal's maxillary dentition.



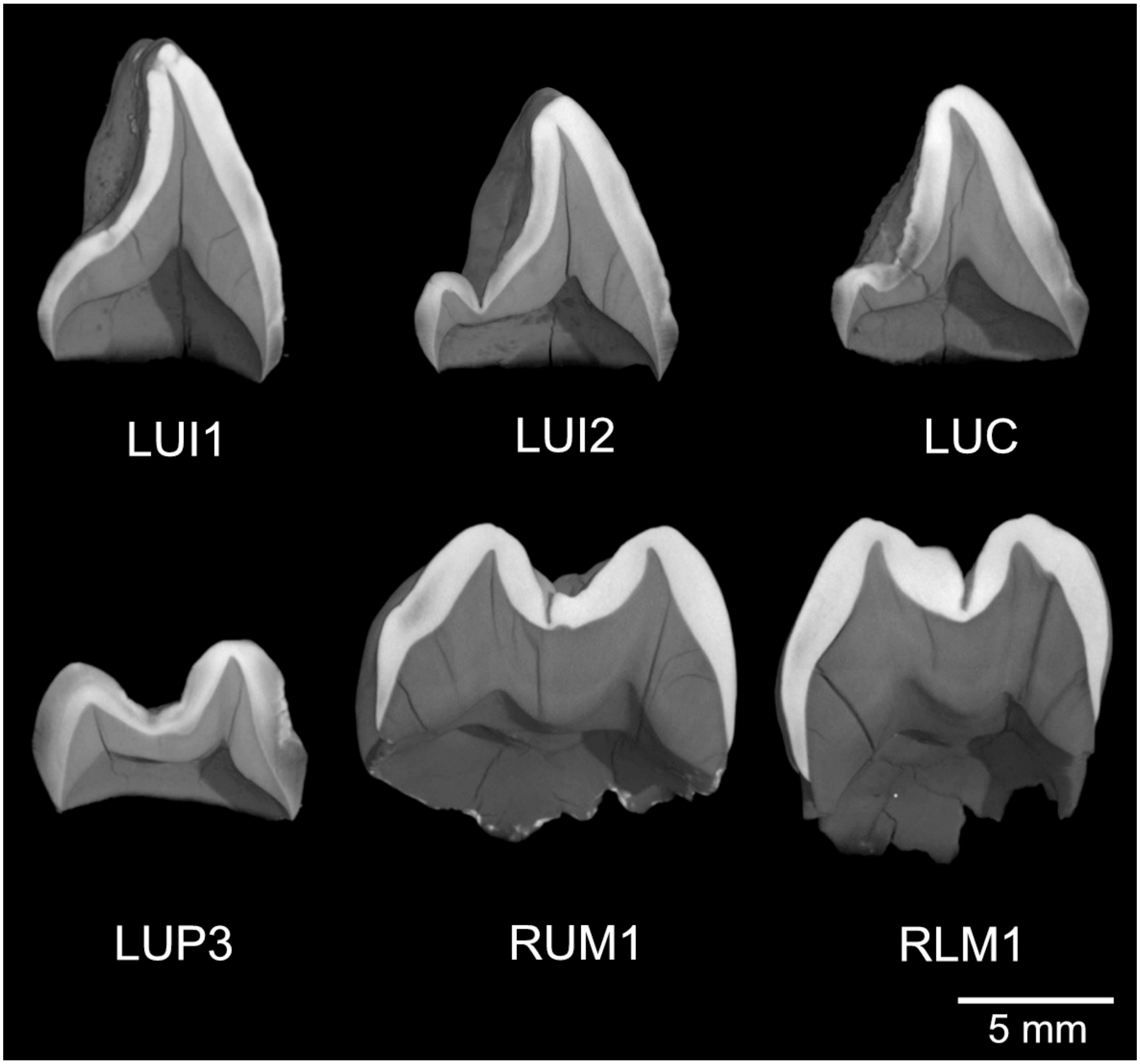
Scanned at the ESRF ID 19 beamline: 31 micrometer voxel size. Note that clinical radiographs of La Quina H18 in (4) were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7).

SI Figure 3. Virtual overview of the Krapina Maxilla B Neanderthal's dentition and associated teeth.



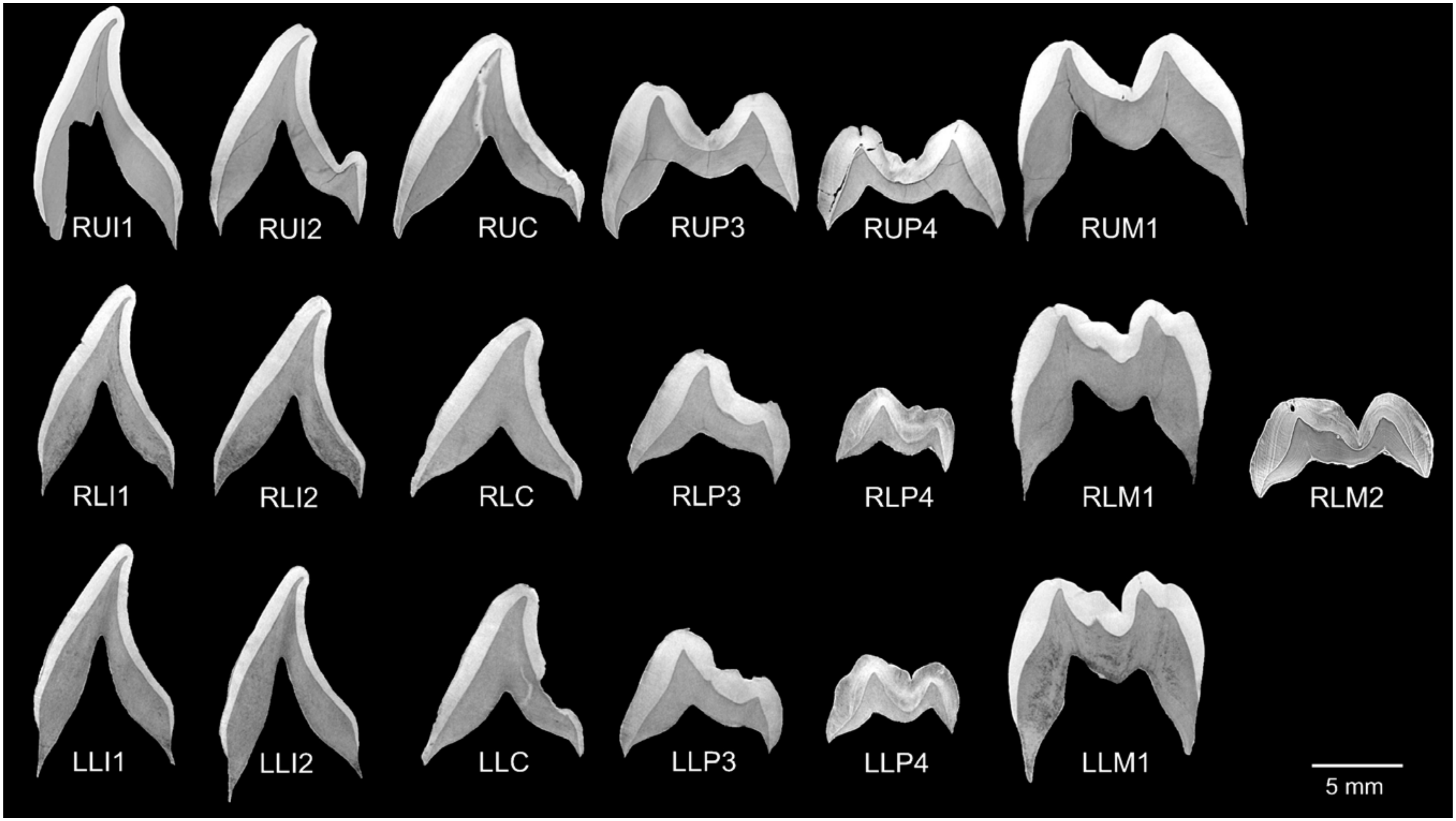
Scanned in Zagreb, Croatia: 14 - 29 micrometer voxel sizes with Skyscan and BIR scanners, respectively. Associated teeth include isolated teeth and those erroneously glued into Krapina Maxilla C (K191, K192, K194, K195, K196). Note that clinical radiographs of Krapina Maxillae B & C, and K191 & K192 in (5) were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7).

SI Figure 4. Virtual overview of the Engis 2 Neanderthal's dentition.



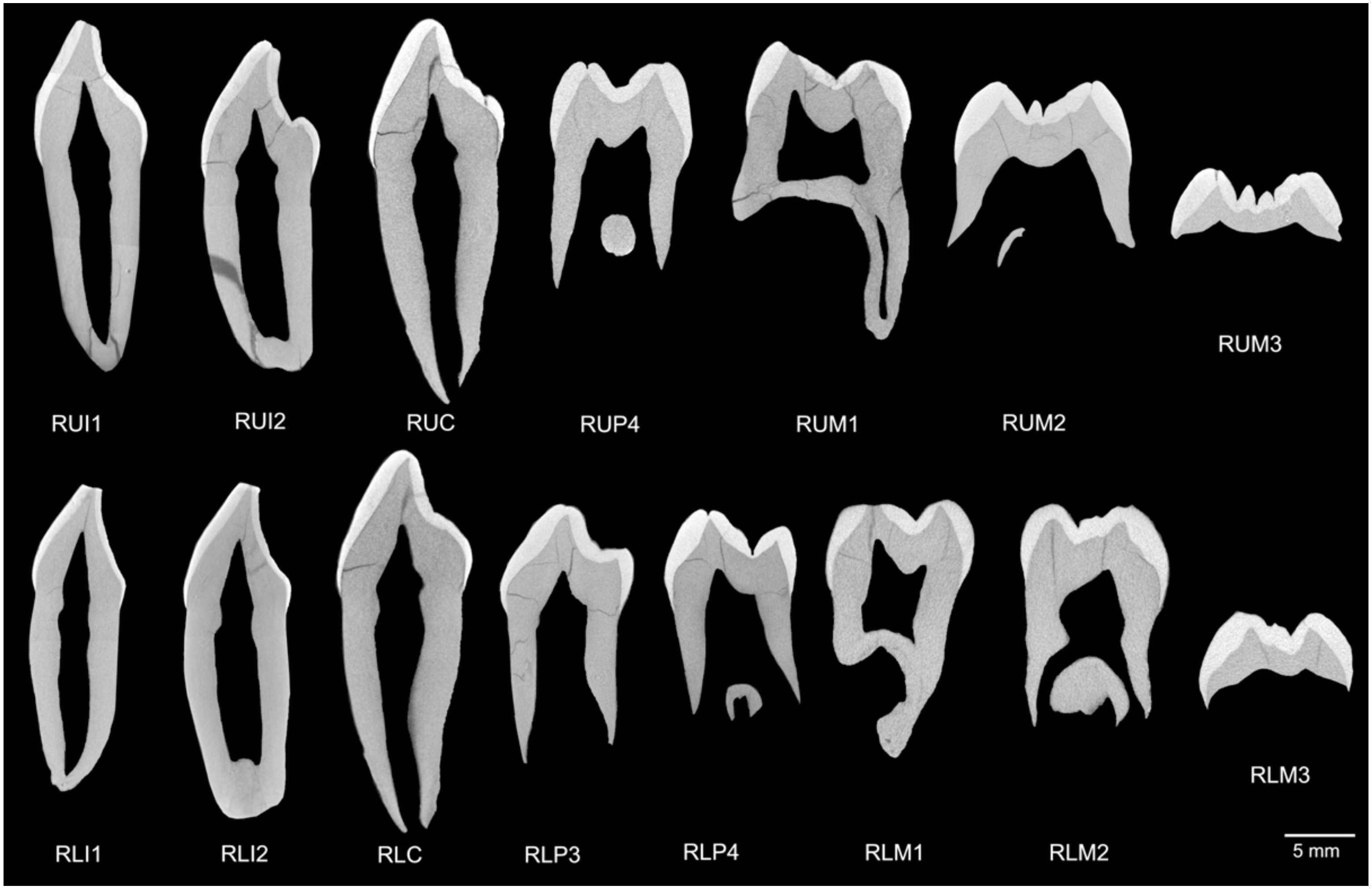
Scanned at the ESRF ID 19 beamline: 31 micrometer voxel size (rendered here in three-dimensions). Note that clinical radiographs of Engis 2 in (4) were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7).

SI Figure 5. Virtual overview of the Gibraltar 2 Neanderthal's dentition.



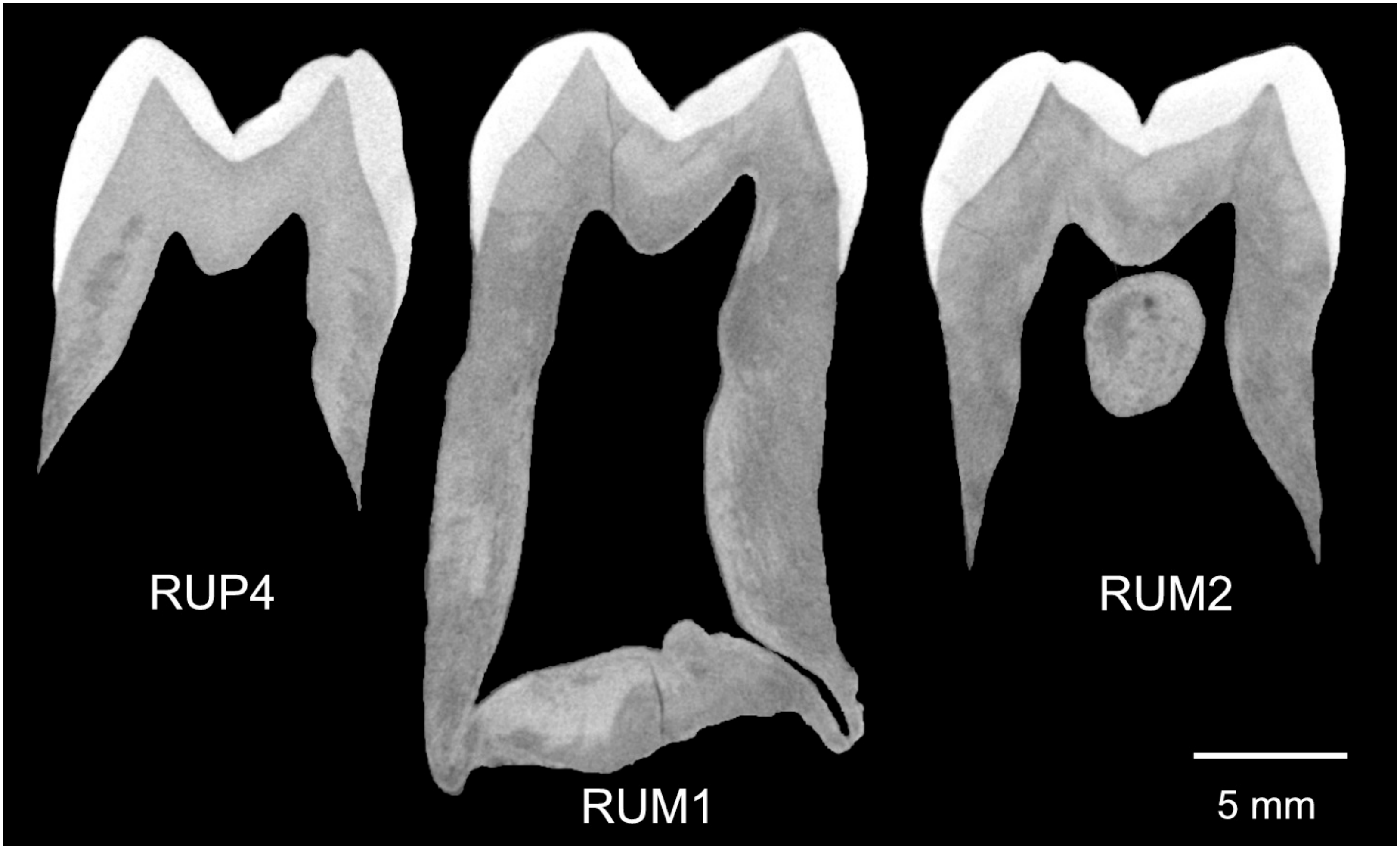
Scanned at the ESRF ID 19 beamline: 31 micrometer voxel size, save for the RLM2, which was scanned at 5 micrometer voxel size with phase contrast. Note that clinical radiographs of Gibraltar 2 in (4) were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7), and the developmentally-advanced left mandibular incisors (I1 & I2) were used for the calculation of predicted recent human age.

SI Figure 6. Virtual overview of the Scladina Neanderthal's dentition.



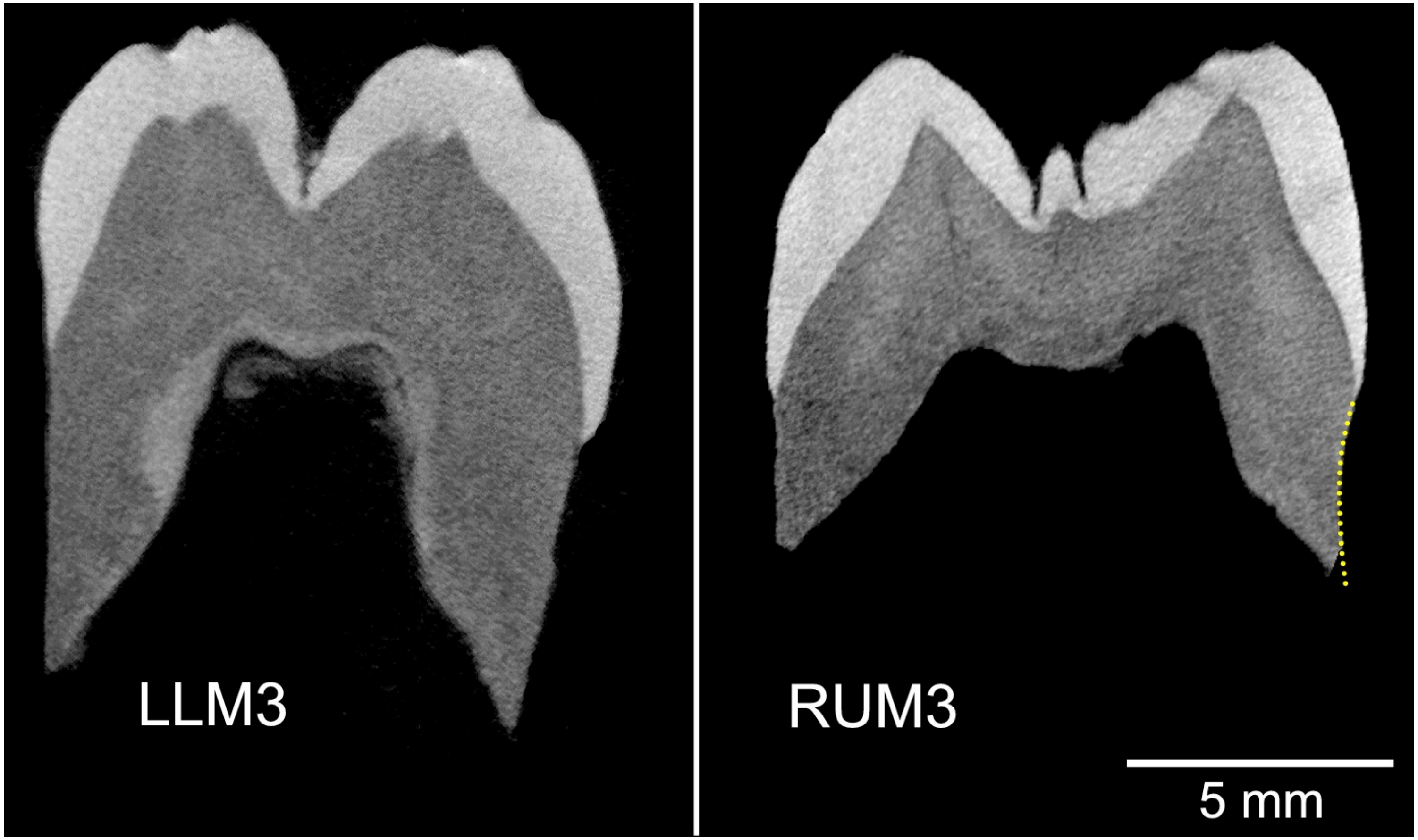
Scanned at the MPI-EVA: 14 - 29 micrometer voxel sizes with Skyscan and BIR scanners, respectively. Because clinical radiographs of Scladina were unavailable, the developmental status was assessed from isolated teeth and micro-CT slices in keeping with the recent human radiographic comparative sample (SI Table 7).

SI Figure 7. Virtual overview of the Krapina Maxilla C Neanderthal's dentition.



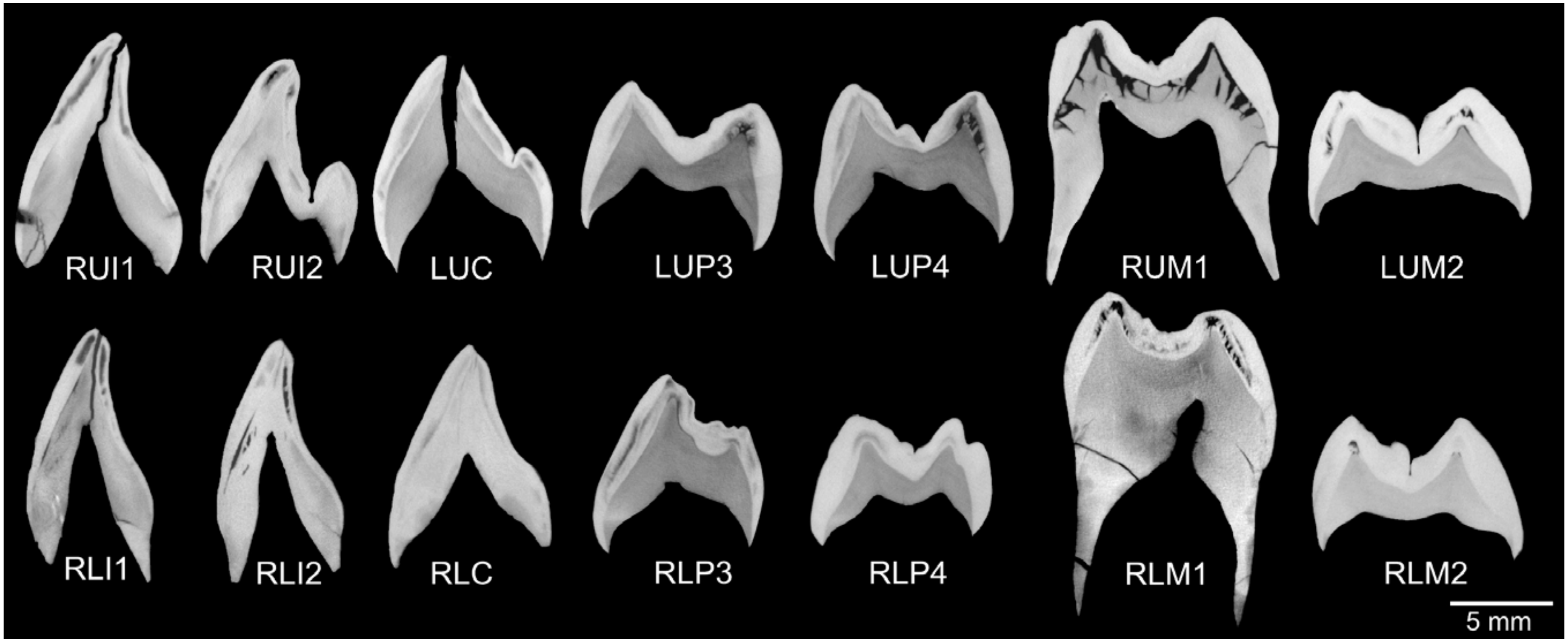
Scanned in Zagreb, Croatia: 29 micrometer voxel size with a BIR scanner. The M1 shows extreme taurodontism, while the *in situ* M2 shows a "foreign body" inside the pulp that is a concretion of mineralized tissue that may be a "false pulp stone." Note that clinical radiographs of Krapina Max C in (5) were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7).

SI Figure 8. Virtual overview of Le Moustier 1 Neanderthal's third molars (M3s).



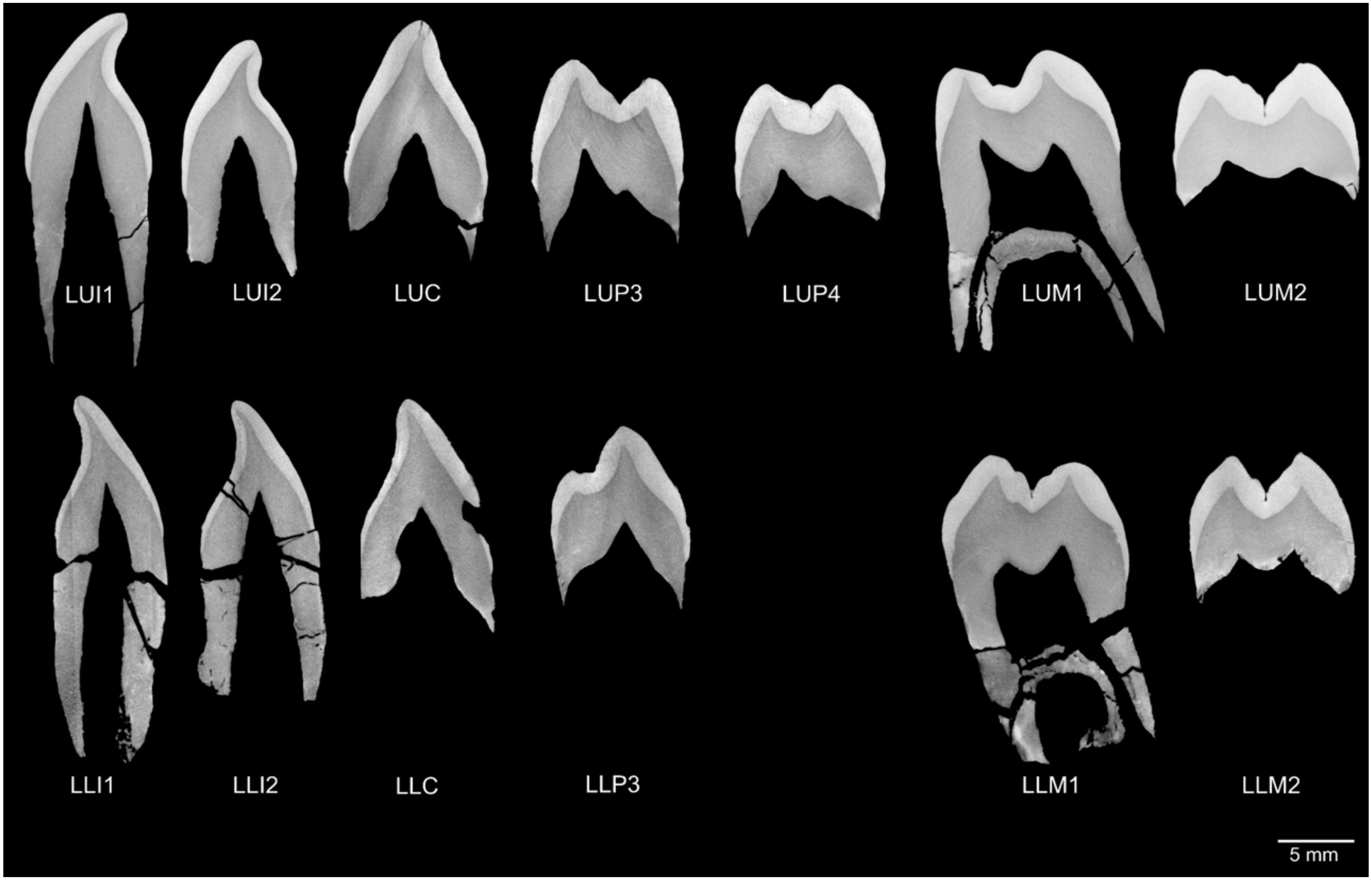
Scanned at the MPI-EVA: 31 micrometer voxel size with a BIR scanner. The dotted line on the mesiobuccal root of the maxillary M3 indicates the root length used to determine the age at death (SI Table 8). Note that digital radiographs taken prior to micro-CT scanning of Le Moustier 1 and micro-CT slices were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7). Our predicted recent human age of 15.8 years at death is nearly identical to Thompson and Nelson's (6) age of 15.5 years derived from clinical CT scans and the same scoring method (7).

SI Figure 9. Virtual overview of the Qafzeh 10 *Homo sapiens* dentition.



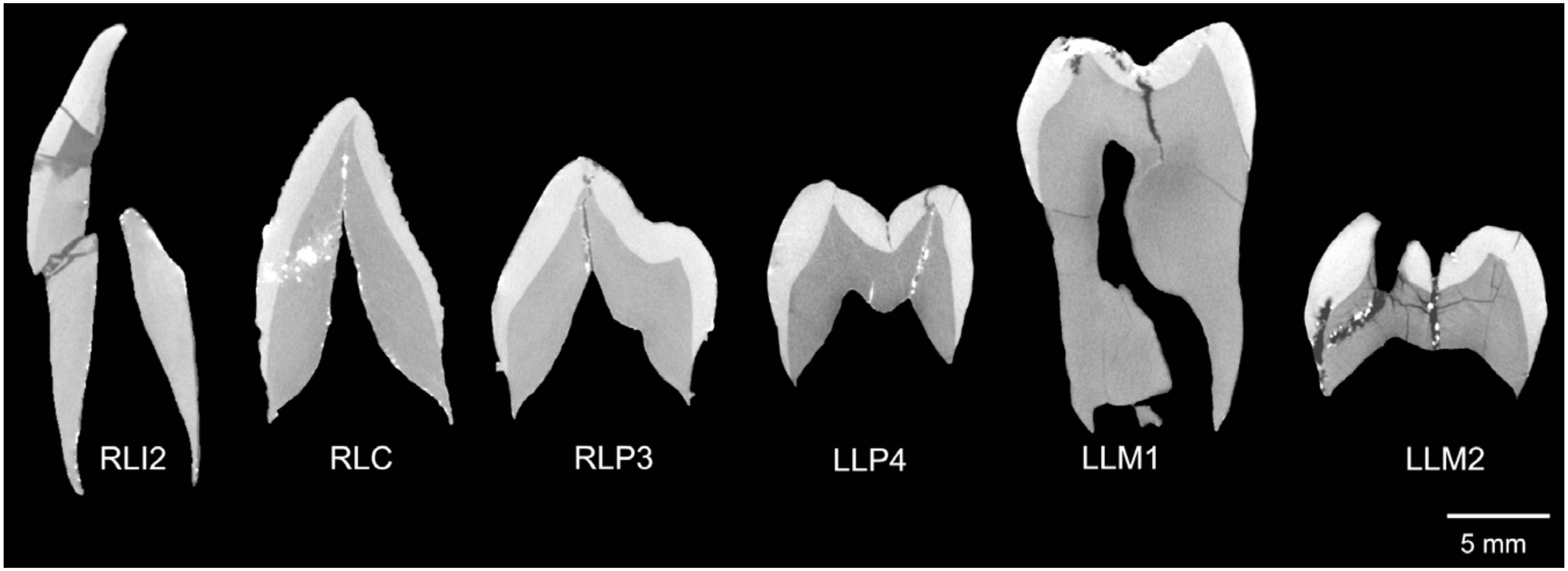
Scanned at the ESRF ID 19 beamline: 31 micrometer voxel size. Note that clinical radiographs of Qafzeh 10 in (8) were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7).

SI Figure 10. Virtual overview of the Qafzeh 15 *Homo sapiens* dentition.



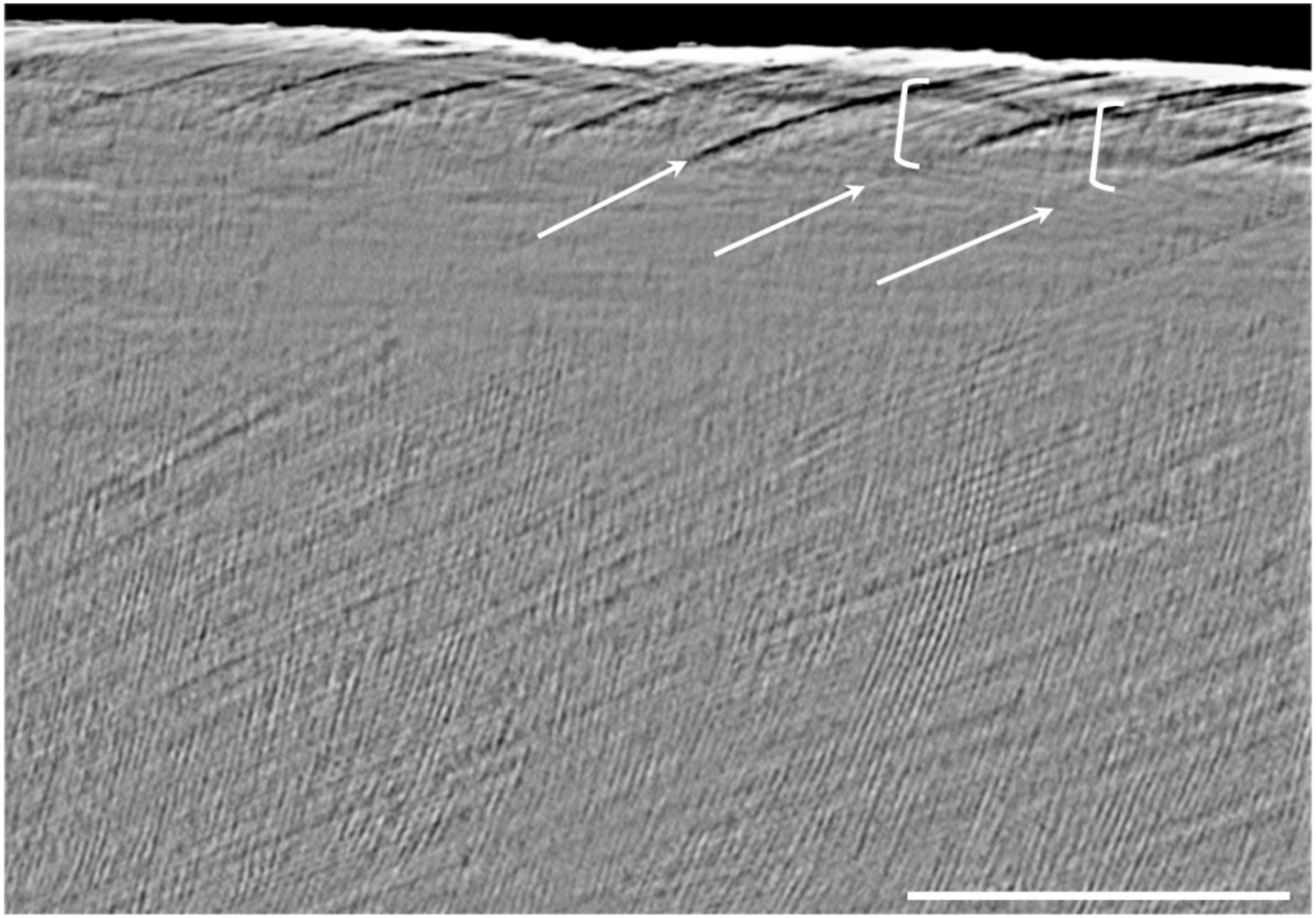
Scanned at the ESRF ID 19 beamline: 31 micrometer voxel size. Note that clinical radiographs of Qafzeh 15 in (8) were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7).

SI Figure 11. Virtual overview of the Irhoud 3 *Homo sapiens* dentition.



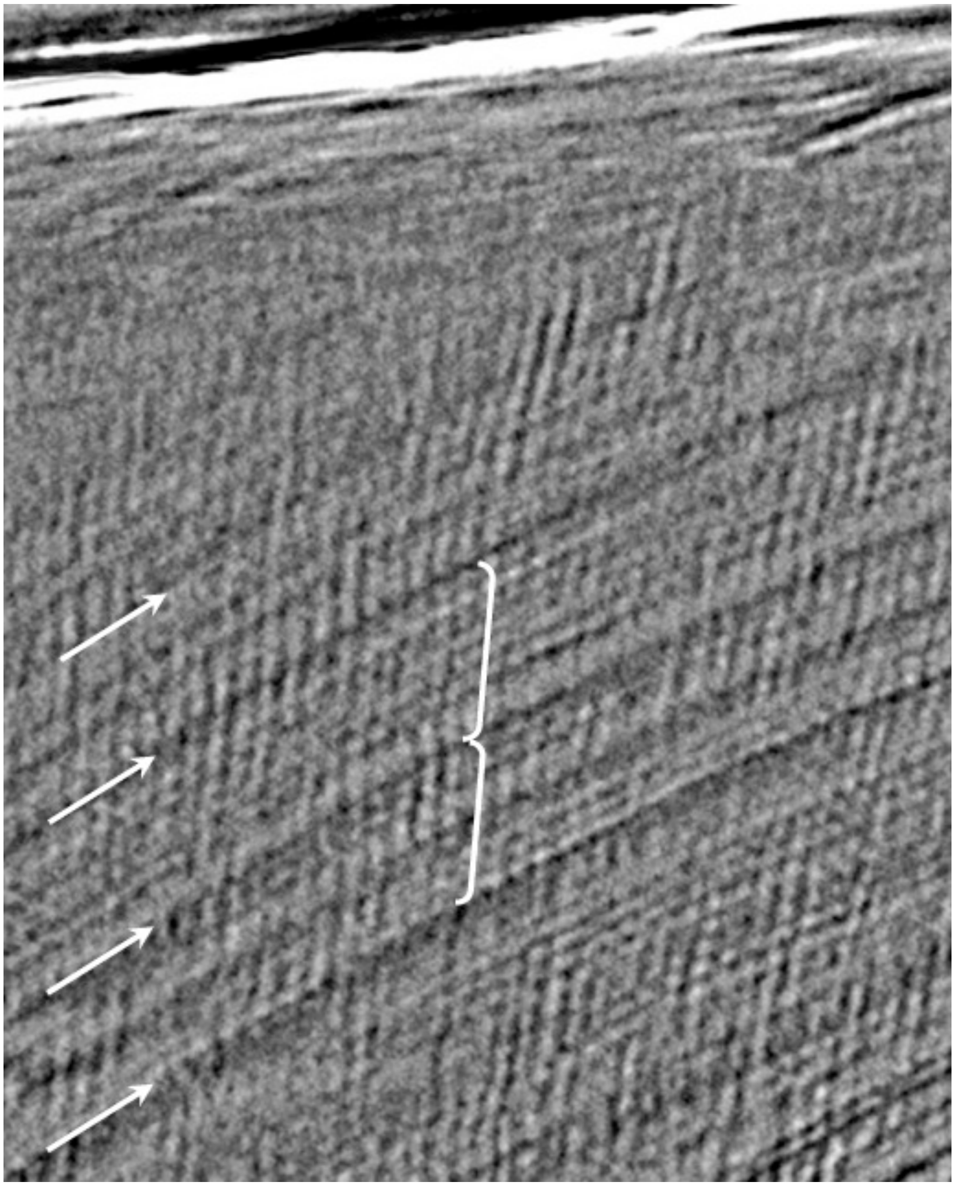
Scanned at the MPI-EVA: 24 micrometer voxel size with a BIR scanner. Note that low resolution micro-CT models of Irhoud 3 in (9) were used to score the developmental status of these teeth in keeping with the recent human radiographic comparative sample (SI Table 7).

SI Figure 12. Eight day long-period line periodicity in the Engis 2 Neanderthal.



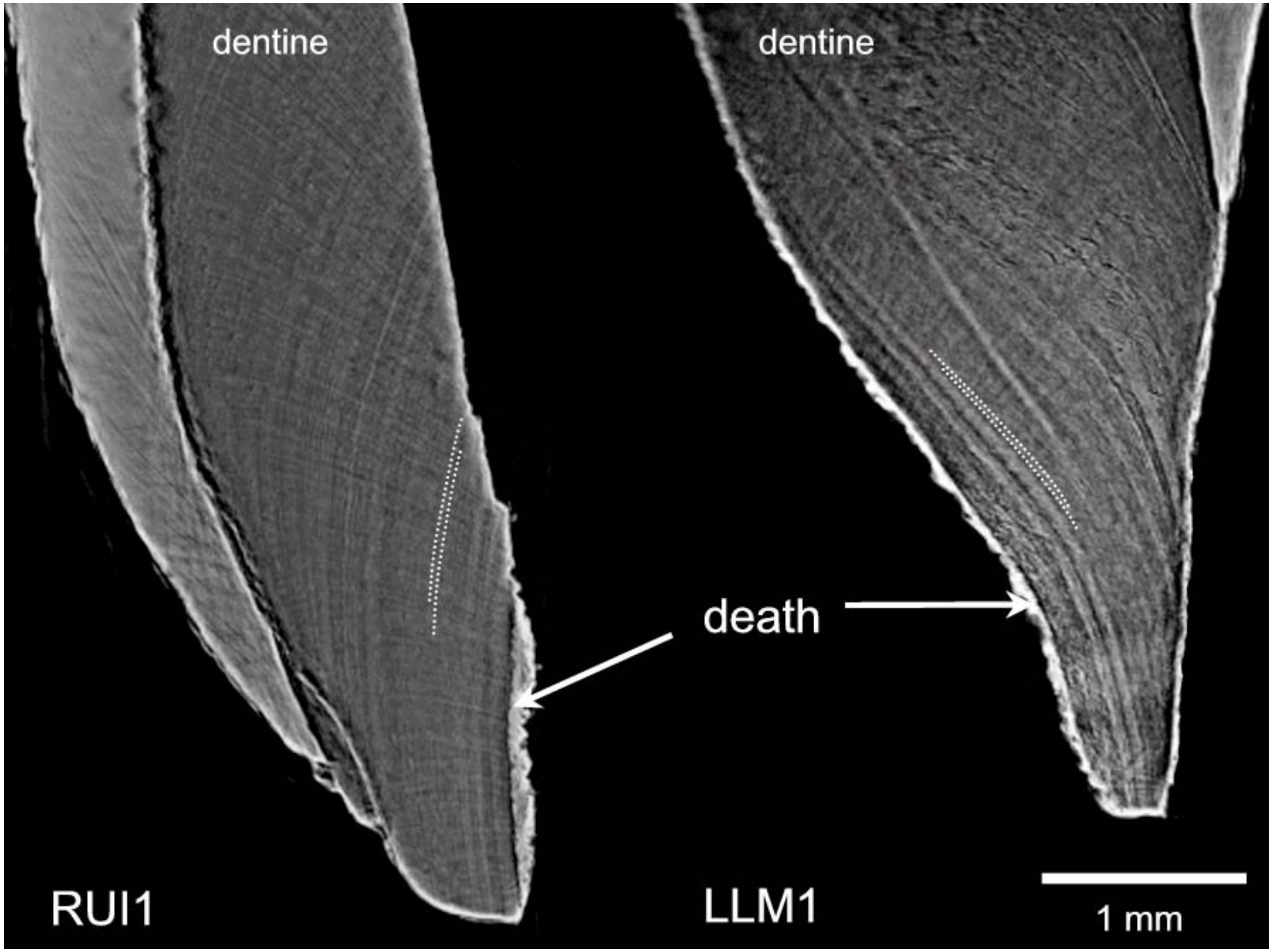
Synchrotron phase contrast image (0.678 micrometer voxel size); 8 short-period lines (in brackets) can be seen between long-period Retzius lines (arrows). In this image, daily laminations (10, 11) were counted as it was not possible to resolve enamel prisms showing cross-striations between Retzius lines. Enamel prisms are vertically oriented, the surface of the tooth is at the top of the image, and the cervix is towards the left. Faint horizontal bands in the upper aspect of the image are artifacts due to inhomogeneities in the X-ray optic (multilayer monochromator) for high resolution imaging. Scale bar is equal to 0.2 mm.

SI Figure 13. Eight day long-period line periodicity in the Le Moustier 1 Neanderthal.



Synchrotron phase contrast image (0.7 micrometer voxel size); arrows indicate long-period Retzius lines, and brackets define the interval containing 8 short-period lines (paired light and dark bands), demonstrating that the long-period line periodicity of this individual is 8 days. Enamel prisms are oriented vertically, the surface of the tooth is at the top of the image, and the cervix is towards the left.

SI Figure 14. Long-period (Andresen) lines in the Gibraltar 2 Neanderthal's root dentine used to calculate age at death.



Long-period lines in the dentine (running parallel to the developing surface - orientation indicated with dotted lines) were counted to determine the root formation time. After hypoplasias and accentuated lines were used to register these two teeth, postnatal crown and root formation time were summed to determine the age at death.

SI Figure 15. Overview of the Obi-Rakhmat 1 Neanderthal dentition.

Because clinical radiographs or micro-CT scans of Obi-Rakhmat were unavailable, the developmental status was assessed from isolated teeth in keeping with the recent human radiographic comparative sample (SI Table 7).

Supporting Tables

SI Table 1. Average cuspal enamel thickness (in micrometers) in Neanderthals, fossil *Homo sapiens*, and recent *H. sapiens*.

Tooth	Cusp	Neanderthals		N	Fossil *H. sapiens*		N	Recent *H. sapiens*		N
UI1	-	737	(690-836)	4	900	--	2	840	(750-960)	11
UI2	-	800	(750-894)	5	795	(625-965)	2	788	(700-900)	16
UC	-	749	(668-872)	5	1098	(895-1300)	2	1056	(750-1250)	25
UP3	b	803	(601-915)	5	1242	(1175-1310)	2	980	(700-1200)	14
UP4	b	1053	(775-1250)	6	1448	(1410-1485)	2	924	(650-1200)	24
UM1	mb	755	(455-1012)	4	985		1	1069	(700-1300)	13
	ml	1027	(860-1259)	4	1212	(1155-1270)	2	1521	(1100-2200)	14
UM2	mb	1255	(1055-1475)	7	1627	(1535-1720)	2	1322	(1000-1600)	23
	ml	1365	(1075-1742)	5	2012	(1855-2170)	2	1688	(1400-2000)	17
UM3	mb	1337	(1105-1568)	5	--			1594	(820-2350)	68
	ml	1455	(1198-1700)	5	--			1855	(1206-2285)	55
LI1	-	610		1	782	(625-940)	2	682	(520-800)	10
LI2	-	695		1	668	(570-790)	3	604	(550-695)	8
LC	-	652	(645-660)	2	812	(695-885)	3	1045	(800-1175)	13
LP3	b	760	(690-830)	2	1148	(1045-1210)	3	937	(600-1300)	19
LP4	b	954	(915-1032)	3	1470	(1345-1595)	2	1112	(950-1350)	16
LM1	mb	834	(612-1447)	7	1153	(1080-1280)	6	1093	(600-1600)	20
	ml	913	(698-1263)	7	1247	(800-1730)	6	1061	(750-1600)	27
LM2	mb	1115	(1060-1185)	3	1730	(1615-1845)		1452	(1200-1700)	32
	ml	1112	(965-1260)	2	1708	(1485-1880)	3	1209	(1000-1550)	25
LM3	mb	1007	(905-1097)	3	~1400		1	1618	(950-2076)	43
	ml	954	(783-1133)	6	~1350		1	1342	(900-1800)	30
	db	1175		1	~1690		1	2190	(1680-2800)	8

Tooth= upper (U), lower (L), central/first incisor (I1), lateral/second incisor (I2), canine (C), third premolar (P3), fourth premolar (P4), and first, second, and third molars (M1, M2, & M3). Postcanine values determined from buccal (b), mesiobuccal (mb), or mesiolingual (ml) cusps for premolars and molars, respectively. Additional samples of Neanderthals and fossil *H. sapiens* are from (1, 9, 12); averages and ranges are shown. Recent human populations (13-15) were pooled as few significant differences are found among populations. Samples showing light attrition were corrected based on the curvature of wear facets and/or adjacent Retzius lines; moderate to heavily worn teeth were excluded.

SI Table 2. Mann-Whitney *U* test results for cuspal enamel thickness differences between Neanderthals and recent humans.

Row	**I1**	**I2**	**C**	**P3**	**P4**	**M1mb**	**M1ml**	**M2mb**	**M2ml**	**M3mb**	**M3ml**
Max											
Z	-2.095	-0.255	-3.327	-1.584	-1.562	-2.161	-2.666	-0.814	-2.474	-2.099	-2.948
P	**0.040**	0.842	**0.000**	0.130	0.129	**0.032**	**0.005**	0.441	**0.011**	**0.034**	**0.001**
Mand											
Z	--	--	--	--	--	-2.010	-2.069	--	--	--	-3.276
P						**0.048**	**0.039**				**0.000**

Row = maxillary (Max) or mandibular (Mand). Tooth = central/first incisor (I1), lateral/second incisor (I2), canine (C), third premolar (P3), fourth premolar (P4), and first, second, and third molars (M1, M2, & M3). Molar teeth are represented by mesiobuccal (mb) and mesiolingual (ml) cusps. Comparisons were made for N > 3. Significantly thinner enamel was found for Neanderthal tooth types where the significance level is in bold. Neanderthal M1 mb cusps are also thinner than fossil *H. sapiens* (*Z*= -2.146, *P*= **0.035**), but this difference is not significant for M1 ml cusps (*Z*= -1.714, *P*= 0.101).

SI Table 3. Long-period line periodicity (in days) in Middle Paleolithic hominins.

Taxon	Individual/Site	Periodicity	Source
Neanderthals	Jonzac	6	This study
	Marillac LUP3	6	" "
	Saint-Césaire 1	7	" "
	Krapina Maxilla B	7	" "
	Lakonis	7	16
	La Chaise BD-J4-C9	7	3
	Tabun C1	8	" "
	Scladina	8	1, confirmed in this study
	Le Moustier 1	8	This study
	Engis 2	8	" "
	Gibraltar 2	9	" "
	Average	**7.4**	
Fossil *H. sapiens*	Hofmeyr	7	This study
	Qafzeh 15	7	" "
	Qafzeh 10	8	" "
	Skhul II	8	" "
	Irhoud 3	10	9
	Average	**8.0**	

Periodicities of Engis 2, Gibraltar 2, Maxilla B, Le Moustier 1, Jonzac, Marillac LUP3, Saint-Césaire 1, Qafzeh 10, Qafzeh 15, Irhoud 3, and Hofmeyr were determined from phase contrast synchrotron imaging. Periodicities of Scladina, Lakonis, La Chaise, Tabun, and Skhul were determined from histological thin sections.

SI Table 4. Average enamel long-period line numbers in Neanderthals and two recent human populations.

Tooth	Cusp	Neanderthals	N	European	N	African	N
UI1		128 (119-138)	4	169 (139-197)	7	121 (110-136)	4
UI2		128 (120-136)	4	131 (107-153)	11	108 (102-116)	5
UC		140 (131-151)	4	148 (100-197)	20	135 (105-169)	5
UP3	b	107 (99-115)	2	122 (108-157)	12	80 (67-94)	2
UP4	b	96 (85-108)	2	107 (79-138)	16	87 (67-100)	8
UM1	mb	82 (74-92)	3	92 (80-124)	8	80 (57-96)	11
	ml	74 (68-79)	2	89 (75-118)	11	87 (70-130)	10
UM2	mb	91 (78-109)	4	85 (65-111)	11	93 (69-120)	13
	ml	79 (77-81)	2	95 (77-112)	6	87 (66-120)	9
UM3	mb	86	1	80 (68-106)	13	92 (64-126)	14
LI1		95	1	134 (113-154)	8	99 (94-104)	2
LI2		114 (101-127)	2	131 (119-152)	5	107 (98-112)	3
LC		154 (140-167)	2	193 (144-249)	12	161	1
LP3	b	106 (94-118)	2	138 (109-182)	17	102 (90-113)	2
LP4	b	103	1	104 (72-135)	10	91 (77-115)	6
LM1	mb	84 (74-90)	3	92 (76-110)	6	90 (82-101)	5
	ml	70 (69-70)	2	67 (51-90)	8	75 (56-90)	18
LM2	mb	78	1	86 (74-103) 1	5	80 (64-99)	6

Tooth= upper (U), lower (L), central/first incisor (I1), lateral/second incisor (I2), canine (C), third premolar (P3), fourth premolar (P4), and first, second, and third molars (M1, M2, & M3). Postcanine values determined from buccal (b), mesiobuccal (mb), and mesiolingual (ml) cusps for premolars and molars, respectively. Samples showing light attrition (missing up to 10% of lateral enamel) were corrected based on the curvature of wear facets, curvature of adjacent Retzius lines, and/or spacing of adjacent perikymata; moderate to heavily worn teeth (more than 10% missing) were excluded. It was not possible to include a number of Neanderthal teeth due to poor surface preservation or the presence of protective surface coating.

SI Table 5. Average coronal extension rates (in μm/day) in Neanderthals and two recent human populations.*

Tooth	Cusp	Neanderthals		N	European		N	African		N
UI1	-	10.42	(9.95-10.89)	2	7.79	(7.23-8.84)	7	6.96	(6.36-7.48)	4
UI2	-	9.86	(8.87-10.84)	2	7.10	(6.02-7.94)	11	5.60	(5.04-6.27)	5
UC	-	9.28	(9.24-9.32)	2	6.52	(5.17-8.00)	20	7.48	(6.73-8.34)	5
UP3	b	8.30		1	5.75	(4.63-6.49)	12	6.49	(6.43-6.54)	2
UP4	b	7.48		1	6.08	(5.16-7.79)	16	6.36	(5.16-7.51)	8
UM1	mb	8.32	(8.32-8.33)	2	6.36	(5.71-7.44)	6	6.23	(4.99-8.07)	6
	ml	8.00	(7.79-8.20)	2	5.94	(5.37-6.59)	7	6.38	(5.56-7.07)	6
UM2	mb	6.11	(5.66-6.45)	3	6.67	(5.89-7.87)	9	5.68	(4.76-6.54)	9
	ml	6.31		1	6.35	(5.22-8.14)	6	5.14	(4.37-6.03)	8
UM3	mb	5.20		1	5.76	(5.11-6.51)	11	5.67	(4.51-6.94)	11
LC	-	8.66		1	6.08	(5.02-7.35)	12	6.89		1
LP3	b	9.10		1	6.15	(5.44-7.29)	17	5.95	(4.89-7.01)	2
LP4	b	8.01		1	6.24	(5.59-7.05)	10	6.27	(5.00-7.19)	6
LM1	mb	8.73	(7.70-10.04)	3	7.34	(6.71-7.72)	5	7.03		1
	ml	8.11	(7.68-8.37)	3	6.62	(5.40-7.48)	4	6.54	(5.45-7.27)	6
LM2	mb	8.28		1	6.14	(5.27-7.29)	13	5.75	(5.52-6.12)	5
LM3	db	5.63		1	4.14	(3.72-4.79)	4	--	-	

Tooth= upper (U), lower (L), central/first incisor (I1), lateral/second incisor (I2), canine (C), third premolar (P3), fourth premolar (P4), and first, second, and third molars (M1, M2, & M3). Postcanine values determined from buccal (b), mesiobuccal (mb), and mesiolingual (ml) cusps for premolars and molars, respectively. Values for LM1 mb and ml cusps include data for La Chaise (3).

* An expanded global human sample of molar teeth (17) was employed for Figure 2.

SI Table 6. Average crown formation times (in days) in Neanderthals and two recent human populations.

Tooth	Cusp	Neanderthals	N	European		N	African		N
UI1	-	1218 (1170-1266)	2	1582	(1500-1645)	7	1318	(1288-1343)	4
UI2	-	1134 (1088-1181)	2	1427	(1354-1558)	11	1324	(1276-1386)	5
UC	-	1240 (1228-1241)	2	1613	(1510-1745)	20	1438	(1409-1466)	5
UP3	b	1159	1	1407	(1313-1467)	12	1113	(1108-1118)	2
UP4	b	1148	1	1249	(1107-1359)	16	1086	(966-1214)	8
UM1	mb	834 (811-857)	2	1017	(957-1074)	6	981	(829-1043)	6
	ml	872 (872-873)	2	1159	(1038-1332)	7	1059	(967-1158)	6
UM2	mb	1059 (947-1176)	3	1018	(939-1144)	9	1145	(1008-1238)	9
	ml	969	1	1141	(1085-1229)	6	1218	(1097-1311)	8
UM3	mb	997	1	1040	(906-1133)	11	1079	(993-1130)	11
LC	-	1305	1	2004	(1810-2111)	12	1721		1
LP3	b	984	1	1454	(1352-1614)	17	1157	(1135-1179)	2
LP4	b	1080	1	1245	(1038-1432)	10	1165	(1072-1276)	6
LM1	mb	941 (825-1041)	3	1097	(1043-1148)	5	1096		1
	ml	846 (788-885)	3	948	(910-980)	4	917	(842-990)	6
LM2	mb	932	1	1096	(1039-1203)	13	1145	(1092-1184)	5
LM3	db	982	1	1288	(1150-1445)	4	--	-	

Tooth= upper (U), lower (L), central/first incisor (I1), lateral/second incisor (I2), canine (C), third premolar (P3), fourth premolar (P4), and first, second, and third molars (M1, M2, & M3). Postcanine values determined from buccal (b), mesiobuccal (mb), and mesiolingual (ml) cusps for premolars and molars, respectively. Values for LM1 mb and ml cusps include data for La Chaise (3). Samples showing light attrition were corrected and included as detailed in SI Tables 1 & 4, while moderate to heavily worn teeth were excluded.

SI 7 Table. Fossil sample tooth formation stages as defined on a scale of 1-14 in (7).

Individual	**Row**	**I1**	**I2**	**C**	**P3**	**P4**	**M1**	**M2**	**M3**	**Recent Human Age**	**Actual Age**
H. sapiens											
Qafzeh 10	max	7	6	6	6	6	10	5		5.1	5.1
	mand	8	8	6	6	5	10	5			
Irhoud 3	mand	11	10	8	8	7	12	6		6.7	7.8
Qafzeh 15	max	11	10	8	9	8	11	7		6.9	unknown
	mand	11	10	8	9		12	7			
Neanderthals											
Engis 2	max	6	6	5	5		7			4.0	3.0
	mand						8				
Gibraltar 2	max	7	7	6	6	5	9			4.8	4.6
	mand	8	8	6	5	4	9	4			
La Quina H18	max	9	7	7	7	6	11	5		5.7	unknown
Krapina Maxilla B	max	9	8	7	7	6	11	6		5.9	5.9
Obi Rakhmat	max		11	9	9	9		9		7.8	6.0-8.1
Krapina Maxilla C	max					9	14	10		9.3	unknown
Scladina	max		14	13		10		9	5	10.5	8.0
	mand	14		13	11	10	14	10	5		
Le Moustier 1	max	14	14	14	14	14	14	14	9	15.8	11.6-12.1
	mand	14	14	14	14	14	14	14	10		

Row = maxillary (max) or mandibular (mand) dentition. Tooth = central/first incisor (I1), lateral/second incisor (I2), canine (C), third premolar (P3), fourth premolar (P4), and first, second, and third molars (M1, M2, & M3). Each tooth was scored independently from radiographs and micro-CT slices several times, average scores were calculated (given here), and converted into recent human ages for each tooth using Tables 1-4 in (7). Predicted recent human ages were calculated as an average of western European male and female mean ages for each available element. There are no available data on non-European populations for comparison.

SI Table 8. Age at death calculation for the Le Moustier 1 Neanderthal (times in days save for the last column).

Initiation Age	Crown Form. Time	Root Formation Time @ 3.5 µm/day	@ 3 µm/day	Age at Death Min days	Max days	Years
2155	997	1086	1267	4238	4419	11.61-12.11

Initiation age estimated from the Scladina Neanderthal maxillary M3 (1). Crown formation time for the maxillary M3 mesiobuccal (mb) cusp was determined as the sum of cuspal and lateral formation, determined from the micro-CT scan, high-resolution cast, and synchrotron virtual histology. A corresponding root length of 3.8 mm was measured from the mb root (SI Fig. 8). Root formation times, calculated as root length divided by root extension rate, are estimated using two different root extension rates. These include a fast-forming model (3.5 µm/day: measured from counts of Andresen lines of known periodicity in the equivalent root length and tooth type of a recent African individual), or a slow-forming model (3.0 µm/day: average value similarly determined from two recent European individuals) as there are no available data on root extension rates in Neanderthal M3s. Age at death was calculated as the sum of initiation age, crown formation time, and root formation time (yielding minimum and maximum values depending on the root extension rate employed). Synchrotron imaging revealed extensive accentuated lines in the cervical aspects of the maxillary incisor and canines, and a number of hypoplasias were also identified on casts. However, it was not possible to register (cross-match) later-forming teeth using hypoplasias or accentuated lines. Decohesion of the X-ray beam (strong scattering due to microporosity) in the root and in the plaster restorative material prohibited imaging dentine microstructure.

SI Table 9. Age at death calculation for the Krapina Maxilla B Neanderthal (times in days save for the last column).

Inititiation Age	**Crown Form. Time**	**Root Form. Time**	**Age at Death Days**	**Years**
102	1238	798	2138	5.86

Initiation age estimated from the Scladina Neanderthal maxillary canine (1), which was validated by registering the anterior teeth of this individual and estimating the initiation ages of the incisors. The Krapina Maxilla B I1s were estimated to have initiated at 42 and 43 days of age, nearly identical to the independent initiation age of the Gibraltar 2 maxillary I1 at 44 days, justifying the use of 102 days for canine initiation. Crown formation time for the canine is the sum of cuspal enamel formation and lateral enamel formation, determined from the micro-CT scan, high-resolution cast, and synchrotron virtual histology. Root formation time is the number of Andresen lines in root dentine multiplied by the long-period line periodicity, determined from synchrotron virtual histology of the crown. Age at death was calculated as the sum of initiation age, crown formation time, and root formation time.

SI Table 10. Age at death calculation for the Obi-Rakhmat 1 Neanderthal.

Max Teeth	**Initiation Age**	**Cusp Time**	**Pkg**	**CFT (7)**	**CFT (8)**	**Prd**	**RT (7)**	**RT (8)**	**Death 6 day**	**Death 7 day**	**Death 8 day**	**Death 9 day**
I2												
lingual	205	224	130	1134	1264	194	1358	1552	6.50	7.39	8.28	9.16
C	102	210	137	1169	1306	113	791	904	4.96	5.65	6.33	7.02
P3												
buccal	617	225	99	918	1017	123	861	984	5.96	6.56	7.17	7.78
lingual	617	322	82	896	978	135	945	1080	6.14	6.73	7.33	7.92
P4												
buccal	750	295	85	890	975	117	819	936	6.18	6.74	7.29	7.84
lingual	750	298	78	844	922	116	812	928	6.06	6.59	7.12	7.65
M1												
mb	-13	211	74	729	803	283	1981	2264	6.41	7.39	8.37	9.35
M2												
mb		352	83	933	1016	110	770	880				
ml		382	81	949	1030	28+	196+	224+				
								Ave	**6.03**	**6.72**	**7.41**	**8.10**

Max Teeth= Maxillary lateral/second incisor (I2), canine (C), third and fourth premolars (P3 & P4), and first and second molars (M1 & M2). For molar cusps: mb= mesiobuccal cusp, ml= mesiolingual cusp. Initiation ages (in days) estimated from the Scladina Neanderthal (1) save for the P4, which was estimated to be intermediate between Neanderthal P3s and recent human P4 data (18). Cusp Time= Average cuspal enamel formation times (in days) of Neanderthals in the current study. Pkg= Perikymata, the number of long-period lines on the enamel surface counted from casts of the original teeth. CFT= crown formation time (in days), calculated as cuspal enamel formation plus lateral enamel formation (total number of perikymata multiplied by Neanderthal long-period line periodicity modal values of 7 and 8). Prd= Periradicular bands, the number of long-period lines on the root surface counted from casts of the original teeth. Slight estimations were made for broken root apices. RT= Root formation time (in days), calculated as the number of periradicular bands multiplied by Neanderthal long-period line periodicity modal values of 7 and 8. Death= age at death (in years) calculated as the age at initiation plus crown and root formation times estimated using the full range of observed Neanderthal periodicity values (6 – 9 days). Age at death was not calculated for the M2 as the mesiobuccal and distobuccal roots were missing, and the mesiolingual and distolingual root lengths appeared to be pathologically foreshortened (19).

Movie Legend S1

Multi-scale synchrotron virtual histology illustrating age at death determination of the Engis 2 Neanderthal. First scale: voxel sizes of 31.3 micrometers in absorption mode for virtual extraction of the full permanent dentition. Second scale: 4.95 micrometers with phase contrast effect for the isolated maxillary M1 (utilized for long-period line counts and observations of the neonatal line). Third scale: 0.678 micrometers with phase contrast for the small cube of enamel utilized to determine the long-period line periodicity.

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